SOON CHUN HYANG UNIVERSITY HOSPITAL CHEONAN

# In Vitro and In Vivo Evaluation of Epidermal Growth Factor (EGF) Loaded Alginate-Hyaluronic Acid (AlgHA) Microbeads System for Wound Healing

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## Introduction

- **Background** : Skin serves as a protective barrier. Damage to the skin impairs its protective function and requires effective healing methods.
- **Objective:** The study aims to evaluate the effectiveness of epidermal growth factor (EGF) loaded alginate-hyaluronic acid (AlgHA) microbeads for sustained EGF release and enhanced wound healing.

## Methods

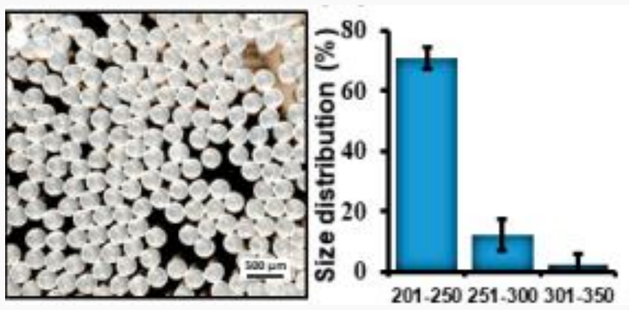
- **Fabrication of Beads**: AlgHA beads were prepared using sodium alginate and hyaluronic acid, cross-linked with heparin.
- **Characterization**: SEM, EDS, bead size distribution, and FT-IR used to analyze bead properties.
- **EGF Release Study**: Loading and release profile of EGF in AlgHA beads.
- **Biocompatibility**: Tested using L929 cells through MTT assay and fluorescence microscopy.
- **In Vitro Wound-Healing Assay**: Scratch assay to evaluate cell migration.
- **Immunoblotting**: Protein expression analysis in rbMSCs.
- **In Vivo Study**: Wound healing evaluated in rat models with histological and immunohistochemical analysis

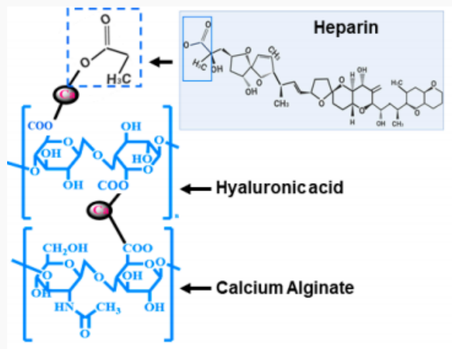
### Key Steps

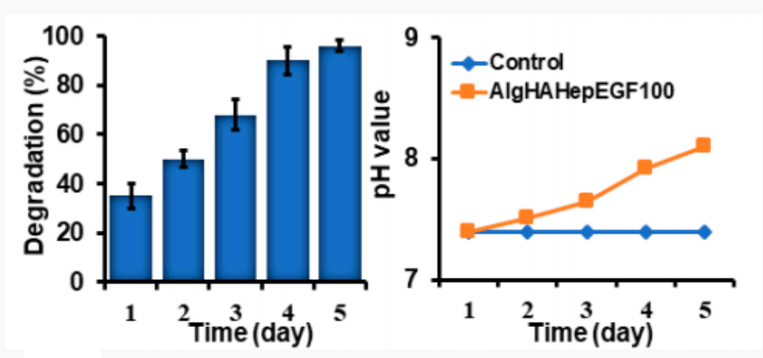
1. Preparation and characterization of AlgHA beads
2. EGF loading and release study
3. Biocompatibility testing with L929 cells
4. In vitro wound-healing assay using scratch method
5. Protein expression analysis via immunoblotting
6. In vivo wound healing study in rats

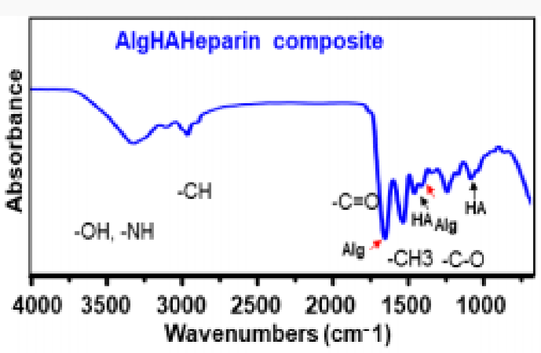
**Table 1.** Preparation of AlgHA beads.

| Beads Type | Alginate (2%) (*w/v*) | Hyaluronic Acid (2%) (*w/v*) | Heparin |
|---|---|---|---|
| AlgHAHep | 80 mL | 20 ml | 5 IU/mL |
| AlgHA | 80 ml | 20 ml | No heparin added |



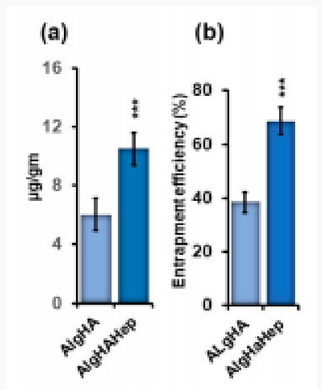




## Results

- **Characterization**: Beads were homogeneous, appropriate size distribution, and confirmed composition.
- **EGF Release**: Controlled release over 5 days, better performance with heparin-crosslinked beads.
- **Biocompatibility**: L929 cells showed significant growth and proliferation.
- **Cell Migration**: Enhanced migration in AlgHAEGF100 and AlgHAEGF150 groups.
- **Protein Expression**: Significant expression of Flk-1 and ICAM-1.
- **Wound Healing**: Improved wound closure and new tissue formation in vivo.



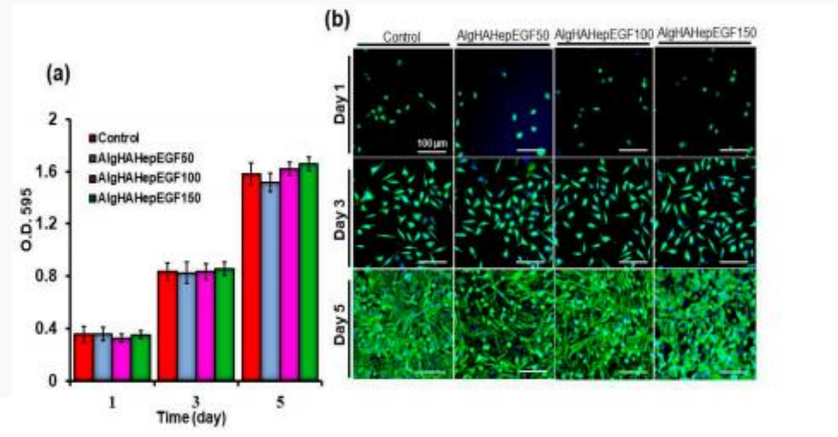
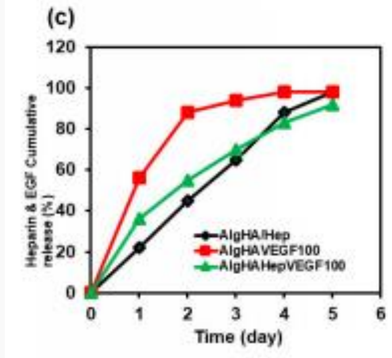




Figure 4. Biocompatibility testing of L929 with the AlgHA scaffold groups by MTT (a) after 1, 3, and 5 days of treatment and (b) nucleus fluorescence microscopic analysis of the L929 cells for cell proliferation by Hoechst staining (Scale bar 500 μm).



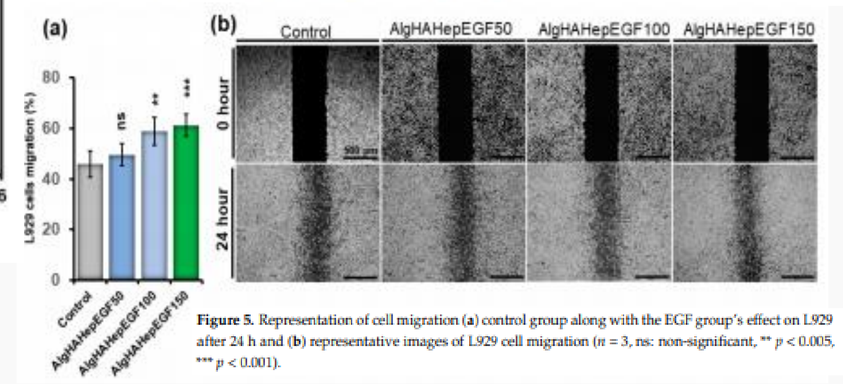
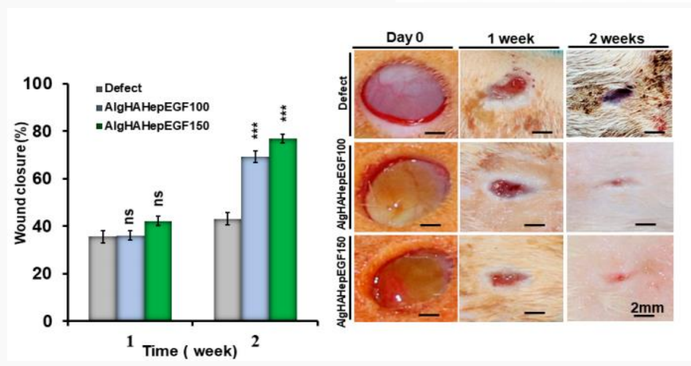




Figure 5. Representation of cell migration (a) control group along with the EGF group's effect on L929 after 24 h and (b) representative images of L929 cell migration ($n = 3$, ns: non-significant, ** $p < 0.005$, *** $p < 0.001$).




## Discussion

- **Effectiveness of AlgHA Beads**: Discusses how the EGF-loaded AlgHA microbeads promote wound healing through sustained release.
- **Heparin's Role**: Highlights the importance of heparin in enhancing EGF retention and controlled release.
- **Clinical Implications**: Potential application in clinical settings to reduce the need for frequent dressing changes.
- **Limitations**: Discusses limitations and suggests future research directions.

## Conclusion

- **Overall Findings**: The EGF-loaded AlgHA microbeads effectively promote wound healing by providing sustained EGF release, enhancing cell proliferation and migration, and improving tissue regeneration.
- **Future Potential**: The system could significantly benefit clinical wound care by reducing the frequency of dressing changes.

**Reference →**